

## ***Toxoplasma gondii* antibodies in domiciled cats from rio branco Municipality, Acre State, Brazil**

### **Anticorpos anti-*Toxoplasma gondii* em gatos domiciliados no Município de Rio Branco, Acre, Brasil**

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#### **Abstract**

Blood samples were collected from 89 cats to assess the prevalence of IgG antibodies against *Toxoplasma gondii* by indirect immunofluorescence (IIF) and the possible risk factors associated with feline *Toxoplasma gondii* infection. An epidemiological questionnaire was developed and implemented for owners of domestic cats domiciled in Rio Branco, Acre. The results were statistically evaluated with the odds ratio and chi-square tests, considering the significance level of 5%. Of 89 animals' samples, 22 had antibodies against *Toxoplasma gondii*. Among the 22 reactive animals, 15 (68.19%) were female, 15 (68.19%) were less than one year old and 20 (90%) were cross breed. Concerning risk factors, there was no difference ( $p > 0.05$ ) between the variables evaluated by the chi-square test. Moreover, 16 (72%) cats were fed a mixed diet, 20 (90%) of the cats had hunting habits, 18 (81%) had contact with animals of another species, 11 (50%) had access to the street, and 21 (95%) lived in homes that had areas of grass or dirt. In conclusion, the prevalence of anti-*Toxoplasma gondii* in domestic cats was 24.7%, and there were no significant risk factors for feline toxoplasmosis in the municipality of Rio Branco, Acre.

**Key words:** Toxoplasmosis, feline, seroprevalence

#### **Resumo**

Com o objetivo de avaliar a ocorrência de anticorpos IgG contra *Toxoplasma gondii* por meio de Imunofluorescência Indireta (RIFI) e associar com os possíveis fatores de risco para a enfermidade, foram colhidas amostras de sangue da veia jugular de 89 gatos domiciliados, na cidade de Rio Branco, Acre após a realização de inquérito epidemiológico pautado nos principais fatores de risco para a presença de anticorpos. Os resultados encontrados foram avaliados estatisticamente com o teste de razão de chances (Odds ratio) e qui quadrado, considerando-se o nível de significância de 5%. Dos animais positivos 15 (68.19%) eram fêmeas, 15 (68.19%) possuíam idade superior a 12 meses e 20 (90%) eram animais sem raça definida. Sobre os possíveis fatores de risco, não houve diferença ( $p > 0,05$ ) entre as variáveis avaliadas pelo teste de qui quadrado. Adicionalmente, 16 (72%) gatos eram alimentados com dieta mista, 20 (90%) mantinham hábitos de caça, 18 (81%) tinham contato com animais de outra espécie,

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11 (50%) tinham livre acesso à rua e 21 (95%) habitavam casas que possuíam áreas de grama ou terra. Conclui-se que na cidade do Acre, Rio Branco, a prevalência de anticorpos (IgG) para anti-*Toxoplasma gondii* nos felinos domiciliados foi de 24,7%, e que os fatores de risco não foram significantes.

**Palavras-chave:** Toxoplasmose, felinos, soroprevalência

## Introduction

Toxoplasmosis is a widespread infectious disease caused by *Toxoplasma gondii*, an obligate intracellular parasite capable of infecting any homeothermic animal, including humans (DUBEY, 2004). This parasite can occasionally be transmitted by ingestion of oocysts present in the feces of felines, known as definitive hosts, after sporulation in the environment or, more commonly, by ingestion of cysts that are located in the muscles of intermediate hosts (DUBEY, 2008).

*T. gondii* has been reported to occur in various regions in Brazil, but with variations, so the risk analysis suggests that there are different local risk factors (CRUZ, 2007). It is therefore of vital importance that each region has its epidemiological study of both prevalence and risk factors, so that the responsible governing bodies responsible can use them as a tool to act on real prophylactic measures for each population (CRUZ, 2007).

In humans, the incidence of congenital toxoplasmosis in Acre from 2002 to 2005 was recorded as 56 newborns of a total of 34,568 evaluated. Thus, the incidence of congenital toxoplasmosis was 1:617, whereas in Rondônia, the same author found a ratio of 1:817 (SERRUYA, 2007).

There is a lack of studies on the prevalence of antibodies against *Toxoplasma* in cats in the state of Acre. According to Braga et al. (2012) evaluation of seroprevalence in animals with access to the outside environment is an interesting alternative to measure the dissemination of the disease, because cats are the bridge between *Toxoplasma* infection in animals and humans. In addition, they reported that the main factor that can increase the rate of infection in felines is the predation of small birds such as pigeons and rodents. This study aimed to

evaluate the occurrence of antibodies to *T. gondii* in domestic cats in Rio Branco, Acre, Brazil, and possible risk factors.

## Materials and Methods

To calculate the sample size for estimating the occurrence of toxoplasmosis in cats, we considered a degree of confidence of 95% and the level of absolute accuracy of the results of 10% (THRUSFIELD, 2004). The expected occurrence of the disease in Rio Branco used in the calculation was 30%, based on the findings of a previous pilot study by the research team in the state. Thus, it would be necessary to sample 81 animals in accordance with the standard formula for simple random sampling (THRUSFIELD, 2004). However, it was possible to sample 89 cats domiciled in Rio Branco.

Owners of domiciled cats answered an epidemiological questionnaire that included data such as age, sex, outside environment access, food type, possible contact with rodents or birds, hunting habit, contact with other cats, type of housing, and transport to rural areas. This study was approved by the ethics committee with the process number 23107.011622 / 2013-01 and protocol number 01/2013.

From November 2012 to October 2013, blood samples were collected by jugular venipuncture in cats. The blood samples were immediately placed in test tubes with EDTA, kept in a thermal box containing ice, and transported to the Wildlife Support Laboratory of the Center for Biological Sciences and Nature. In the laboratory, the blood samples were centrifuged at 1000 rpm for 5 min, in order to separate the plasma for subsequent freezing in Eppendorf® brand tubes, which were properly identified and frozen at  $-20^{\circ}\text{C}$ .

The samples were processed using indirect immunofluorescence (IIF) for toxoplasmosis. For this purpose, tachyzoites from the RH strain of *T. gondii* were used as antigen, obtained from an intraperitoneal washing conducted using sterile saline and heparin (25 µL of heparin in 50 mL of saline) of mice on the second day post-infection. These were inactivated by adding 0.5% formaldehyde in an incubator at 37°C for 30 min. After centrifugation to create a pellet containing the peritoneal exudate cells, the supernatant was removed and centrifuged again, and the supernatant obtained was discarded. The pellet was then resuspended in phosphate buffered saline (PBS) and centrifuged again. After resuspension, the pellet was evaluated microscopically for the number of tachyzoites and then diluted in PBS to obtain an average of 20–30 tachyzoites per field. Ten microliters of this tachyzoite suspension were placed into “wells” (printed microscope slides by the silk screen technique). The slides containing the antigen were then maintained at room temperature overnight in order to dry, and were covered to prevent contamination. Subsequently, they were wrapped in aluminum foil and stored at –20°C until use.

The sera from cats were diluted in PBS in multiples of 4 (1:16 to 1:4096). Positive and negative control sera were used for comparison. Titers equal to or greater than 64 were considered positive according to the methodology used by Rosa et al. (2010).

The results of the titration and questionnaire were analyzed statistically using the results of the odds ratio (OR) measure of association and chi-square test, with a significance level of 5%.

### Results and Discussion

Of the 89 animals, 22 (24.7%) were serum positive in IIF for IgG antibodies against *T. gondii*. Among the positive specimens, the titer of 1:64 was more frequent, present in 15 cats, which corresponded to 68% of positive animals (Table 1). These results were different from those of Garcia et al. (1999) in Jaguapitã, Paraná, who found that the most frequent titers in cats from rural areas were 1:4096 (28.6%) and 1:256 (21.8%).

**Table 1.** Serological results of indirect immunofluorescence (IIF) in domiciled cats tested for IgG against *Toxoplasma gondii* according to age group, in Rio Branco, Acre.

Age group	n	1:64	1:256	1:1024	Positive	NR.
Young (<12 months)	42	6	1	0	7	35
Adult (>12 months)	47	9	5	1	15	32
<b>TOTAL</b>	89	15	6	1	22	67

n: number of samples, NR: non-reactive samples.

With regard to risk factors, chi-square analysis showed no difference ( $p \geq 0.05$ ) in any parameter studied (Table 2), but in the evaluation of OR, 15 positive animals (68%) were older than 12 months; thus older cats had a higher seroprevalence (OR = 2.34) for toxoplasmosis compared to young cats. Pena et al. (2006) found similar results in adult (41.4%) and young cats (13.7%). According to Tasawar et al. (2013), a high prevalence is expected both in young and old animals, due to low resistance

against parasitic infections. However, according to Dubey (2004), the prevalence tends to increase with the age of the animal because of the increased chance of exposure over the years, and is also dependent on the lifestyle of the cat, since semi-domiciled and not domiciled cats tend to hunt more than cats kept inside the house. Thus, older animals are more affected by the disease, because they have a greater chance of already having had previous contact with the parasite (ROSA et al., 2010).

**Table 2.** Risk factors for the presence of antibodies in domiciled cats tested for IgG against *Toxoplasma gondii* in Rio Branco, Acre.

Risk Factor	ODDS RATIO	SIGNIFICANCE LEVEL
<b>Older than 12 months</b>	2.34	p > 0.05
<b>Sex – Female</b>	2.76	p > 0.05
<b>Breed – without defined breed</b>	2.18	p > 0.05
<b>Mixed diet</b>	1.69	p > 0.05
<b>Access to outside environment</b>	1.03	p > 0.05
<b>Hunting habit</b>	0.81	p > 0.05
<b>Habitat with grass area or soil</b>	1.69	p > 0.05
<b>Contact with other species</b>	0.5	p > 0.05

Of the 89 cats with tested sera, 54 were females and 35 were males; 15 of the positive cases (68.19%) were females and 7 (31.81%) were males; therefore, females were 2.76 times more likely to have antibodies to toxoplasmosis than males. In addition, according to the OR, males over 1 year of age had a 6.92 times more chances of positivity for antibodies against *T. gondii* than young males. In their study, Tasawar et al. (2013) observed a higher prevalence in males than in females, suggesting that the females have higher levels of immunoglobulins such as IgG and IgM. However, this difference was not observed by Rosa et al. (2010) or Garcia et al. (1999). In a survey of 282 cats in the city of Curitiba, Paraná, Cruz et al. (2011) reported that there was no difference between positive and negative cats in terms of sex and age ( $p > 0.05$ ), which corroborates the results obtained in Rio Branco, Acre.

Of the 22 positive cats, 11 (50%) had free access to the outside environment. Rosa et al. (2010) found a correlation between seropositive reactions and animals that frequently went outside the house. In the city of Porto Alegre, Rio Grande do Sul, Pinto et al. (2009) reported that with regard to access to the street, 47.11% (49/104) of cats were positive by IIF, demonstrating a high value compared to those who did not have outside access. Cats that have free access to the outdoors are more susceptible to *T. gondii* infection because they have the opportunity to hunt small prey and have access to household waste, which may contain meat remains infected by the parasite (BRAGA et al., 2012).

Of the positive animals, 20 (90%) were mongrel and were 2.18 times more likely (OR) to be seropositive for toxoplasmosis compared to the group of animals with defined breed, which, according to Moura et al. (2009), along with free access to the outside environment, may confer a risk factor due to greater exposure to sources of infection. This difference can be explained by the fact that cats with a defined breed are normally exposed to fewer risk factors, such as diet and access to the outdoors.

Regarding diet, it was revealed that 16 (72%) positive animals were fed a mixed diet consisting of food and meat. Animals fed a mixed diet had a 1.69 times increased chance (OR) of having antibodies against *T. gondii* compared to the group of animals that were fed only pet food. Although this feature is known to be an important risk factor, Rosa et al. (2010) found no significant difference in the prevalence of infection in relation to diet. In contrast, Silva et al. (2002) suggested that the high prevalence is because cat owners feed their animals with poorly cooked meat.

Of the 22 positive animals, 20 (90%) kept hunting habits. The hunting habit increased by 0.81 times the chance of presenting titles for toxoplasmosis when compared to animals that do not have this habit. This result corroborates with the findings by Cruz (2007), who reported that risk factors for cats in the epidemiological survey did not differ between the compared groups, including hunting behavior.

Twenty-one (95%) cats inhabited houses that had areas of grass or dirt, and living in shelters without total floor covering increased by 1.69 the risk of toxoplasmosis (OR = 1.69), but this was not significant ( $p > 0.05$ ). This finding was similar to those reported by Rosa et al. (2010) and Moura et al. (2009), who failed to correlate toxoplasmic infection with the type of floor.

The 24.7% prevalence of seropositivity of this study was higher than that observed in the city of Niterói/RJ, which was 19.5% (GONÇALVES NETTO et al., 2003), and in Curitiba/PR, which was 16.3% (CRUZ, 2007), and lower than the prevalences found in São Paulo, which were 27.8% and 23.6% (LUCAS et al, 1998, 1999).

In the northern region, seropositivity was found in higher levels in animals from rural areas. In the municipality of Monte Negro, Rondônia, the occurrence of antibodies against *T. gondii* was 79.4 % among cats from rural areas (CAVALCANTE, 2001). In contrast, in an agglomeration of a native population in Manaus, also in the Amazon region, the frequency of seropositivity in cats was 90.6% (FERRARONI; MARZOCHI, 1980). These data show the need for studies aimed at animals not domiciled and from rural areas, where the feeding of raw meat scraps and hunting habits are more frequent.

Regarding animals in contact with other species, 18 (81%) seropositive animals had this characteristic; therefore, contact with other species increased the chance of toxoplasmosis seroprevalence by 0.5-times when compared to the group of animals that had no contact with other species. Moura et al. (2009), despite not having found differences in their study, point out that cohabitation between dogs and cats may represent a significant risk factor.

The variables of gender, breed, access to the outside environment, age, diet, hunting habits, habitat with grass area or soil, and contact with animals from other species were not associated with seropositivity of animals.

## Conclusions

The prevalence of antibodies (IgG) against *Toxoplasma gondii* in domiciled cats was 24.7%. It should be emphasized that the risk factors were not significant for the presence of antibodies in Rio Branco, Acre, but the most relevant ones were sex (OR = 2.76), age (OR = 2.34) and breed (2.18), followed by housing in homes with direct access to areas of grass and soil (OR = 1.69), mixed diet (OR = 1.69), and hunting habits (OR = 0.81).

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